THE PROBABLE BACTERIAL ORIGIN OF THE GUM OF LINSEED MUCILAGE.

(*Bacilli lini, i.-ii.*, n.spp.)

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That the production of gum or slime in plants may be due to bacteria, can be readily understood in those cases in which the production is evidently abnormal such as the gummosis of the Sugar-Cane and the gum-flux of certain members of the Leguminosæ, Rosaceæ, etc. But when the formation of gum, slime or mucilage is a normal or presumably normal feature of a plant as in Tilia, Linum and the Quince, it is not so easy to believe that the origin may be bacterial. Still, since it has been proved by the author that many of the vegetable gums have a bacterial genesis there is the possibility that the vegetable mucilages, which are after all but varieties of gum, may be the products of bacteria even when these occur normally in plants.

I have examined the tissues of the Lime, Quince and Linseed bacteriologically, and in all cases I have found slime-forming bacteria, but as Linseed is perhaps the most important economically, I shall in this paper deal with it.

It is a matter of common knowledge that the seed of *Linum usitatissimum* contains practically no starch, and that the digestible carbohydrates consist chiefly of mucilage. Much of the mucilage is found as a layer on the outer surface of the seeds, and when these are soaked in water the mucilage swells but does not to any extent dissolve. It may be removed mechanically and a slime obtained.

According to Andes,* Linseed mucilage is dextrorotatory and insoluble in Schweitzer's reagent (ammonio-copper hydrate). When boiled with 1·25% sulphuric acid it is converted into cellulose and a gum. Further boiling produces a sugar. The gum is indifferent to iodine and is dextrorotatory. The most recent work upon the chemistry of the slime is that of Hilger,† who found that the crude slime after extraction with hydrochloric acid to remove the mineral constituents was dextrorotatory. The concentrated aqueous solution gave insoluble compounds with copper sulphate, Fehling's solution, mercury salts and basic lead acetate; neutral lead acetate formed a compound only upon warming. The purified slime was starch-free and contained, besides a little ash, about half a per cent. of cellulose. With nitric acid it yielded mucic acid, and with hydrochloric acid, furfural. From the analysis and the estimation of the amounts of mucic acid and furfural he devised the formula for the gum $2(C_6H_{10}O_5).2(C_5H_8O_4)$. When hydrolysed with 0·5-1 % sulphuric acid, it yielded a mixture of galactose, dextrose, arabinose and xylose as shown by a method devised by him for the detection of these sugars.

I have prepared many samples of the gum from the mucilage by soaking the whole seed as well as the meal overnight in water and pressing the mucilage through calico and thereafter separating the cellulose by subjecting the mucilage, acidified preferably with sulphuric acid, to a pressure of three atmospheres in the autoclave. The cellulose was thus separated, though not so easily as could have been wished, and the solution of gum was precipitated with alcohol and afterwards made into a thick mucilage with water. The tests were made with drops of the mucilage and drops of reagent. The reactions of the several samples, purchased from different stores in Sydney, are given in tabular form.

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* Gummi Arabicum und dessen Surrogate, Leipzig, 1896, p.162.

† Berichte der Deut. Gesell. 36, 3197.

The Reactions of Samples of Linseed Gums.

	1	2	3	4	5 (meal)	Hilger
Alcohol	†	†	†	†	†	†
Fehling's sol.	†	†	†	†	o	†
Basic lead acetate	†	†	†	†	†	†
Neutral lead acetate	?	?	†	?	†	?
Barium hydrate	?	†	†	†	†	–
Copper sulphate	o	o	o	o	o	†
Ferric chloride	o	o	o	o	o	–
Phosphotungstic Acid ...	?	†	†	†	†	–
Tannic acid	†	?	†	†	?	–
Silver nitrate	o	o	?	?	o	–
Mercuric chloride	–	o	o	o	–	†

†, A coagulation or a precipitate; ?, an opalescence or a slight precipitate; o, no reaction ; –, not tested.

These tests are sufficient to show that samples of Linseed yield gums which behave differently with chemical reagents, and from this it must be inferred that the gum is to a certain extent a variable product.

From a perusal of Hilger's paper, there would appear to be no difficulty in obtaining the sugars. He used sulphuric acid, the strength of which lay between 0·5 and 1 %. I have used the same acid in strengths between 1 and 5 % and have boiled the solution for times varying from 2 to 30 hours in order to preclude any doubt that the hydrolysis had not been complete. The experiments showed that the gum is easily hydrolysed and that the products of hydrolysis, while reducing Fehling's solution, are chiefly non-saccharine. The bulk of the reducing substances yield those indefinite osazones that were obtained in the case of Hakea gum. Out of many examinations I have only obtained one definite osazone-galactosazone, and that was present in comparatively small amount.

These indefinite osazones are converted by the further action of phenylhydrazine solution or of dilute acetic acid into brown

or black tarry bodies with a low melting point. It is all a question of the time that the solution remains on the water-bath as to whether one obtains a yellow indefinite osazone or a brown tar. In one case the gum was hydrolysed with 1 % acid, and half of the neutralised solution was heated with phenylhydrazine and acetic acid for three hours on the water-bath. Little more than tar was formed. The second half was heated carefully for thirty minutes after each addition of reagent, and a quantity of osazone-like bodies was obtained. The first fraction consisted of a buff-coloured powder which consisted of a mixture of osazones readily soluble in ether. This solvent was used for fractionating the mixture, and portions were obtained melting at 130°, 139°, 141°, 145° and 149°. The mixture probably consisted of two osazones melting about 130° and 150°. All the other fractions, which were more or less dark in colour, were added together and treated with (1) ether [twice], (2) hot water [twice], and (3) cold alcohol [thrice]. When the quantity justified, the fraction was further split up with ether or cold alcohol. In this way the osazone product was resolved into a tarry substance and into yellow osazones melting about 130°, 150°, 170°, and 193°. The last was galactosazone.

The above is an example of other examinations, and it is evident that the gum is similar in its nature to the exuded gum of *Hakea saligna*.* The only point of difference is that in Linseed gum there is a component that hydrolyses to galactose, while in Hakea gum the nearest allied body furnished an osazone melting at 190°.

Had Linseed gum been capable of giving dextrose or arabinose upon hydrolysis, the osazone of either sugar would have been detected with comparative ease. These sugars are not decom-

* Since writing the above, I have seen an abstract (Biochem. Cent. iii. 1904, 225) of a paper by Lemeland upon the gum of *Cochlospermum gossypium*, DC. It was hydrolysed with difficulty by 1 % sulphuric acid, producing pentoses (proved by the formation of furfural), galactose and intermediate bodies. It appears to be much the same kind of gum as that of *Hakea saligna* and of Linseed mucilage.

posed to any extent by boiling 5 % acid, and their osazones are not destroyed when heated with dilute acetic acid, for I have repeatedly obtained their osazones during my researches in connection with the bacterial origin of the gums. It would appear that the method devised by Hilger for the recognition or separation of arabinose, glucose, galactose and xylose is not capable of distinguishing between the first two sugars and substances that may be closely allied. Glucosazone is one of the easiest osazones to isolate, and it undoubtedly was not among the products of hydrolysis of the gum.

With the object of examining the tissues of the plant to determine the nature of the gum that might be produced by the bacteria contained therein, I obtained several plants from the Hawkesbury Agricultural College, and these were subjected to the usual bacteriological process for the separation of the individual organisms. The bacteria were, comparatively speaking, very numerous, and the majority were capable of producing slime upon media containing saccharose or levulose. The organisms were found not only in the capsules but also in the stems, where they preponderated. There were many kinds, but those that appeared to be similar were grouped together, and from each group one organism was selected. These were gradually narrowed down to what appeared to be three species. There were many races of these species, and that race which seemed to produce the most slime was taken as the representative of the species.

Obviously the first thing to be done was to obtain the gums from these bacteria in sufficient quantity for testing so that they could be compared with the gum from the natural mucilage. It is here that the indefinite nature of Linseed gum is found to be so troublesome. The only reactions that were constant were obtained with alcohol, basic lead acetate and tannin. As the first two reagents coagulate practically every gum, they are of little value for the recognition of different gums. Then the products of hydrolysis furnish indefinite osazones. In short, it appeared that it would be difficult to trace any relationship

between the natural mucilage and the gum formed by micro-organisms inhabiting the tissues of the plant.

The three bacteria were grown upon the media that assisted them to form a maximum of slime, and from the slimes the bacterial cells were separated as completely as possible by the autoclave method of treatment. The gums were evaporated to mucilages and tested to see how closely they resembled Linseed gum. The reactions are given in the following table, and it will be seen that the organism marked "b" appears to be the most promising. Its reactions are practically those of Linseed gum. The alcoholic precipitate was curdy, and not adhesive when touched like so many of the gums; in this it resembled Linseed gum. The gums were also similar in giving thin, weakly-adhesive mucilages with considerable quantities of precipitate. Furthermore, the gum was rather difficult to obtain from the bacterial slime, a condition suggestive of the not readily decomposible cellulose-gum compound of Linseed mucilage.

THE REACTIONS OF THE BACTERIAL GUMS.

	a	b	b (2)	c	Average Linseed Gum.
Alcohol	†	†	†	†	†
Fehling's solution ...	†	†	†	o	†
Basic lead acetate ...	†	†	†	†	†
Neutral lead acetate ..	o	?	?	o	?
Barium hydrate	†	†	†	†	†
Copper sulphate	o	o	o	o	o
Ferric chloride	o	o	o	†	o
Phosphotungstic acid ...	†	†	†	†	†
Tannic acid	?	?	?	?	†
Silver nitrate	o	o	o	o	o
Mercuric chloride ..	o	o	o	o	o

†, A coagulation or a precipitate; ?, an opalescence or a slight precipitate; o, no reaction.

The gum of organism "c" gave the reactions of arabin and the bacterium had the cultural characters of *Bact. acaciæ*. There

were but few colonies. The column marked "b(2)" shows the reactions given by a second quantity of the gum of organism "b."

Organism "b" was a large-sized spore-forming bacillus and grew moderately well on saccharose-potato-agar, on which it produced a white slimy growth. In the slime the cells appeared to preponderate. The slime, as has been already mentioned, did not easily separate into gum and coagulum. It had to be acidified with several drops of dilute sulphuric acid, which, bearing in mind that it was already acid, meant a stronger acidification than is usually necessary with bacterial slimes. Acidification with tartaric acid did not effect a separation even when the heating in the autoclave was prolonged; this acid had been found very useful during my earlier researches in gum-formation. After being freed from reducing substances, by repeated precipitation from aqueous solution with alcohol, the gum was boiled with 5% sulphuric acid for six hours. Portions of the solution when tested showed the presence of reducing sugars and the absence of gum precipitable with alcohol. The osazones were prepared in the usual manner, and after purification from tarry bodies were separated into galactosazone, and another with a melting point near 170°. The latter was very difficult to separate from the former as, although it was more soluble in alcohol, water and ether, the difference in solubility was not sufficiently pronounced to enable the separation to be easily effected.

Thus the gum formed by the bacterium has been found to contain an anhydride of galactose and another substance which yields an osazone having a melting point near 170°.

Organism "a" was by far the most numerous bacterium in the tissues of the plants, if the colonies that developed on the plates were any criterion. Its slime was more viscous than that of the others, and this would accentuate the relative preponderance on account of the tendency of the cells to aggregate, many giving rise to one colony. The reactions of the gum are very similar to those given by average Linseed gum, the only difference being with neutral lead acetate. Upon levulose-asparagine-agar, with or without tannin, it produced a tough slime which yielded

a relatively thin gum. When hydrolysed, the gum gave off furfural and yielded a solution which reduced Fehling's solution. Treatment of this solution with phenylhydrazine solution gave galactosazone and tarry impurity.

With regard to the tarry impurity, I showed in my work upon Hakea gum that the impurity is formed by the action of the phenylhydrazine solution, or even of dilute acetic acid upon certain indefinite osazone-like bodies which are formed from indefinite reducing substances, probably akin to the furfuroïds of Cross, Bevan and Smith. These are found among the products of hydrolysis of every vegetable gum.* In some cases, as in the natural and artificial product of *Bact. acaciæ*, the definite sugars, arabinose and galactose, preponderate in the products of hydrolysis and the tarry impurity is rapidly separated. In other cases, as in Linseed gum, Hakea gum and the gums of organisms "a" and "b," the definite sugar galactose is in small amount, and the tarry body preponderates to such an extent as to make it evident that the gums are hydrolysed to the furfuroïd bodies chiefly.

As in the case of the gum of *Hakea saligna*, the indefinite nature of the products of the hydrolysis of Linseed gum militate against the possibility of being able to trace the source of the gum to any organism. It must, however, be borne in mind that Linseed mucilage is admitted to consist of a gum and of cellulose. The latter is not generally formed by bacteria (the only known case being that of *Bact. xylinum*, the vinegar bacterium), and it is, therefore, possible that the gum, while being originally produced by a bacterium or by several species of bacteria, is altered by the host-plant into the mucilage which we find.

The arguments in favour of such a view are many. We cannot conclude that the gum has not a bacterial origin, because it does not exude from the plant like other recognised bacterial gums. As a rule, it is only when the plant is surcharged with gum that it exudes from punctures, cracks or other wounds.

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* The so-called galactan gums give the furfural reaction, *e.g.*, the gum of *Bact. sacchari*.

The species of *Sterculia* appear to contain, normally, a mixture of arabin and pararabin gums, and I have shown that two bacteria are responsible for the production of these. In *Hakea saligna* we have a case in which a gum, very similar to Linseed gum, is found oozing from the plant; it differs from Linseed mucilage in containing no cellulose. In the gummosis of the Sugar-cane the gum can be seen only when the canes are cut transversely.

The host-plant can alter the functional activity of the microbe so that a gum of a different chemical constitution is produced. This was noted after Peach trees had been infected with the arabin-former. Metarabin exuded, and in the tissues of the plant were found transition forms between *Bact. acaciæ* and *Bact. metarabinum*, which clearly indicated that the former was being converted into the latter. Although *Bact. acaciæ* was in the tissues of the plant, its product, arabin, was not in the exudate. It was assumed that it had been there, but that it had been washed away by rain. This assumption might have been wrong, for it is just possible that the arabin had been altered directly into metarabin. In *Bact. macrozamiæ* we have an organism that produces the natural gum of Macrozamia and which formed the same gum in the laboratory soon after its isolation. After a time, however, the product altered, a gum with different chemical reactions being formed.

These instances show that the plant has, in some cases, a tendency to alter the bacterial production of the gum. We must believe that there is a reason for this. Either the alteration-product is less noxious or it is more useful. I can hardly incline to the former, because it is difficult to understand how a more insoluble and, therefore, more viscous gum can be less harmful than a limpid variety. It is more likely that the plant finds a use for a limited quantity of the gum and that certain kinds are adapted to certain plants. It is quite possible that the pectin bodies may be in part formed from the gums that have been prepared by bacteria. If the bacterium, which has gained entry into the plant, produces a suitable gum, good and well, but if it does not, the host modifies the functional activity. It may alter

the gum, but the alteration of a bacterial gum while in the tissues of the plant would be extremely difficult to prove, for it is only by the stability of the bacterial product that we are enabled to trace the relationship. Yet it requires no great stretch of imagination to believe that such an alteration of gum is probable. If the transmutation of the carbohydrates and the alteration of oil into carbohydrates are admitted, why not also admit the conversion of bacterial gums?

The alteration of the functional activity of a bacterium raises the question of the relationship of the gum bacteria. Was there originally one species which has become so modified by different plants that it appears as many? The determination of a bacterial species depends largely upon the growth-characters, and as these are influenced by the nature of the matrix in which the cells are imbedded, it follows that the matrix or gum plays an important part in determining the species. No two bacteria could be more different in their cultural characters than *Bact. acaciæ* and *Bact. metarabinum*, yet these are varieties of the same organism, producing gums of different solubility. Another example is *Bac. Atherstonei*, which forms a soluble or an insoluble gum. A difference of colour-character is met in *Bac. pseudarabinus*, which may produce a yellow or a white colony. What may be called the bacteria of the vegetable gums, *i.e.*, those whose gums are hydrolysed to arabinose or galactose, are similar in size, in motility, in flagella, in the absence of spores, in being negative to the Gram stain, and in slowly liquefying gelatine. As it is chiefly, if not only, in the nature of the gum-product and in the characters, which this involves, that the bacteria of this group vary, there is a strong probability that they had one common origin, and that the forms in which we find them, are due to the transmuting action of the host-plants. This group of bacteria contains organism "a." Even before the products of the bacteria had been examined, I was led to the belief from the comparative variety of the races and of the species of bacteria as well as the comparative numbers present in the tissues of the plant, together with the variable reactions of the samples of Linseed gum, that

Linseed mucilage is derived not from one microbe but from many. I may be wrong in my belief, and one bacterium may indeed be responsible. If so, then organism "a" or "b" may be the active microbe. On the other hand, the true producer may not have been isolated.

From the research the following conclusions may be summarised :—

1. The gums of Linseed mucilages vary in their chemical reactions, and therefore probably vary in their chemical constitution.

2. The product of hydrolysis consists of galactose and reducing substances which yield indefinite osazones and which are possibly akin to the furfuroïds of Cross, Bevan and Smith.

3. The gum bacteria in the tissues of Linum are relatively very numerous, and consist chiefly of races of two species.

4. The chemical reactions of the gums from these are practically identical with the reactions of Linseed gum.

5. The gum of one of the bacteria is hydrolysed to galactose, and of the other to galactose and a reducing substance that yields an indefinite osazone. Both gums contain a large proportion of the furfuroïd substances.

6. The gum, formed by bacteria, is probably altered by the plant into mucilage and other substances required in the plant economy.

7. A number of so-called species of gum bacteria have probably one common origin; the host plant can alter the nature of the gum product which influences the growth-characters.

Bacillus lini i., n.sp. (Organism "a.")

Shape, etc.—The bacterium appears as a motile short rod, negative to the Gram stain. On nutrient agar, the cells are thin and measure $0{\cdot}3 : 0{\cdot}6\text{-}1{\cdot}5\,\mu$, the average being $0{\cdot}3 : 1\,\mu$. On saccharose-potato-agar, the cells were stouter and measured $0{\cdot}5 : 0{\cdot}8\text{-}1{\cdot}2\,\mu$, the average being $0{\cdot}5 : 1\,\mu$. In bouillon, they appeared as a mixture of the thin and thick forms. The flagella are numerous and peritrichous; up to five have been observed.

Relation to temperature, etc.—Slime is produced at 22°, and there is no growth under the mica plate.

Glucose-gelatine plate.—The colonies do not grow so freely as many other slime bacteria. They are white and raised and dull with an irregular margin. The centre of the colony is raised above the remainder, and from the base of the raised centre short furrows radiate out half-way to the edge. Microscopically, the structure is coarsely granular, much more so than the colonies of *Bact. acacia*, the granular masses are aggregated in the middle and the margin is practically homogeneous and non-granular. While the surface colonies are yellow and translucent, the deep colonies are circular, brown, and clouded or opaque. By the sixth day, the colonies have become depressed centrally and the colour has altered to pale buff.

Nutrient agar plate.—The white colonies are always thin or flat and semi-transparent: they tend to spread either in a circular or an amœboid manner. Microscopically, the colony is yellowish and transparent, with large loose granules in the centre; the deep colonies are irregular, translucent and granular.

Glucose-gelatine stab.—The growth is filiform, with a flat, sunken white nail-head which becomes broad, semi-transparent and dry, while the centre sinks and the subsurface gelatine becomes liquefied in a napiform manner.

Glucose-gelatine stroke.—It develops as a narrow, white, raised, dry growth with a somewhat lobular margin. The medium under the growth slowly liquefies.

Nutrient agar stroke.—The stroke at first is broad, flat and pale buff, with a smooth edge: the infection line becomes raised and rough from the formation of minute wrinkles.

Saccharose-potato-agar stroke.—The growth is at first, raised, buff and glistening; the edge is smooth and the cross-section angular. The culture spreads out, the margin becomes lobular and the surface contoured.

Potato.—A moist-glistening, raised, buff, spreading growth which deepens in colour to yellow.

Bouillon.—The medium becomes very turbid, develops a loose film and a coherent sediment. Indol is formed and nitrates are reduced to nitrites.

Milk.—The medium becomes slightly acid, but is otherwise unaltered.

BACILLUS LINI ii., n.sp. (Organism "b.")

Shape, etc.—The organism appeared as a large rod, staining deeply but irregularly; the outline of the freshly isolated cells was generally lobular. The dimensions were variable, 1·2-1·5 : 3-10 μ; the average being 1·5 : 5 μ. In bouillon and saccharose media, chains of cells and thread forms were found. The cells were coloured by the Gram method of staining, but a few were decolorised, these being in all probability dead cells. After about nine months' cultivation, the spongy protoplasm of the cell had condensed, the rod stained uniformly and measured 1·2 : 3-6μ. The spores, when mature, were generally oval, measuring 1·2 : 1·7-2 μ; occasionally they were reniform. As a rule they were central, but some were excentric. The microbe was very feebly motile; the flagella were numerous and peritrichous.

Relation to temperature, etc.—Slime is produced at ordinary temperatures. There was practically no growth under the anaërobic conditions that prevail under the mica-sheet in plate cultivations.

Glucose-gelatine plate.—The colonies appeared as circular, white, liquefied areas, and when viewed microscopically were brownish-black and granular like a yeast colony. As the liquefaction of the medium proceeded, the colony consisted of a white spot in a crateriform, liquefied area. The subsurface colonies were very slow to liquefy the gelatine, and appeared flat, white and glistening.

Nutrient agar plate.—The colonies were raised, circular, and of a pale cream colour; the surface was rough, although the sheen was that of a fat. Microscopically, the colour was grey-brown, the centre was opaque and indefinite, the margin clouded and the edge waved, showing the finely granular structure of a yeast colony.

Glucose-gelatine stab.—This was filiform; at the surface, a restricted crateriform area bore a white film. The liquefied area became funicular.

Nutrient agar-stroke.—At first, narrow, white and glistening, the stroke became broad, fat-like, canary-coloured, raised and terraced.

Saccharose-potato-agar stroke.—A broad, raised, translucent white slime was formed. It became mottled, partly transparent, partly translucent. Finally, the culture gravitated to the foot of the vertical slope.

Potato.—There grew scanty, dry, white crusts, which fused and became pale buff, while the medium darkened.

Bouillon.—This remained clear, but produced a flocculent sediment and slight surface ring. The indol reaction was obtained, and nitrates were not reduced to nitrites.

Milk.—This became slowly peptonised and developed an acid reaction.